

REMARKS

The claims have been amended to obviate the rejections made under 35 U.S.C. § 112, paragraphs 1 and 2. The invention does not reside in the particulars of ligating DNA fragments to obtain nucleic acids for the expression of fusion proteins, *but rather in the recognition that the functionality of a functional portion of the fusion protein is retained only if the remainder of the fusion represents a soluble fragment (of an original starting protein).*

Thus, the claims as presently proposed focus on the central concept of the invention – that the behavior of the functional portion of a fusion protein is directly coupled to a particular characteristic of the fused amino acid sequence. The fused amino acid sequence will permit the functional protein portion to retain its function if and only if the remaining amino acid sequence represents a soluble protein when divorced from the fusion. Whether this amino acid sequence is a fragment of an insoluble or soluble protein, it in any case represents a soluble domain.

Thus, by producing this fusion protein, either in host cells, such as *E. coli*, or in a cell-free system, by identifying those fusions that retain the functionality of the functional protein, it can be concluded that the attached amino acid sequence represents a soluble domain – a fragment of the protein which is now soluble even if the starting protein is not. This, then, represents a domain that is worthy of study to analyze *in vivo* functions and search for inhibitors as set forth on page 11 of the specification, lines 12-14.

The claims as presented are supported by the claims as originally presented; they simply limit as active steps assessment of the function of the functional protein. Support for new claims 21-23 is found in former claim 10; digestion just from one of the two possible ends is supported in Figure 1 and Example 2. No new matter has been added and entry of the amendment is respectfully requested.

Formal Matters

Applicants appreciate the acknowledgement of the receipt of formal drawings. The requisite papers and disc in compliance with 37 C.F.R. § 1.821 have been submitted in this case on 23 May 2002. In a telephone conversation with the Examiner, our Legal Assistant has confirmed that this sequence listing has been received and has been entered.

The Rejection Under 35 U.S.C. § 112, First Paragraph

All claims were rejected, apparently based on an asserted lack of adequate written description. Applicants are not entirely clear as to what specific defects need to be addressed. The Office first states that the DNA set forth in the claims is described solely by function and not structure. It is not clear whether this is a basis for rejection. In the event that it is, applicants point out that the method of the invention may be employed using the DNA encoding any starting protein; there is a plethora of sequences associated with such insoluble proteins available in the art. Similarly, DNA encoding various functional proteins is also well known. There should be no need to spell out any specific nucleotide sequence since the method is broadly applicable to any starting protein and any functional protein.

The Office goes on to state that the “essential” method step of ligating the DNA is omitted. The claims presently proposed do not require this as an “essential” step; the ligation will already have been performed when the method steps now claimed are conducted. It is believed that the objection to lack of “essential step” from item (c) to (d) (whatever that essential step might be) referred to at the bottom of page 3 of the Office action is also now inapplicable to the presently proposed claims.

Similarly, as claim 10 has been canceled and replaced by an alternate claim, the objections on page 4 of the Office action appear moot. In particular, it has been clarified that it does not matter the method involves fragments of an insoluble protein or fragments of a soluble

one. The fragments must still be soluble. The particular steps set forth in claim 10 do not reappear as such.

With respect to the criticism of “GFP or derivative thereof” this language has been altered to conform to the specification at page 9, lines 6-13. As noted, there are many known “variants” of green fluorescent protein (“GFP”) of various colors.

All claims were also rejected under 35 U.S.C. § 112, first paragraph, as assertedly lacking enablement. The Office outlines several criteria in judging enablement in which it is asserted the claims fall short. The first is said to be the quantity of experimentation necessary, but the discussion under this heading does not appear to relate to the quantity of experimentation, but rather criticism of claim wording as lacking essential steps. It is not clear what essential steps are omitted, and this is not spelled out. Nevertheless, this basis for rejection has been obviated by amendment to the claims.

The second criterion has to do with the breadth of the claims, the amount of direction or guidance presented and the presence or absence of working examples.

Here, the criticism is apparently solely directed to “an unspecified amount of GFP derivatives.” Again, the Office is referred to page 9 of the specification, lines 6-13, which refer to the art-known existence of a multiplicity of GFP variants. In addition to the documents referred to, applicants point out that a large number of GFP variants in a variety of colors is available commercially and can be purchased from commonly accessed scientific supply houses.

The third factor (labeled IV) is the nature of the invention/state-of-the-art and relative skills of those in the art/predictability or unpredictability of the art.

Again, the basis here appears to be the absence of specifying a ligation step in the claims; this objection has been obviated by amendment.

In view of the foregoing, it is believed that the rejections under 35 U.S.C. § 112, paragraph 1, may be withdrawn.

The Rejection Under 35 U.S.C. § 112, Paragraph 2

This basis for rejection, for the most part appears identical to that set forth under the preceding section. Applicants' response is much the same; this rejection is obviated by amendment to the claims.

The rejection specifically applied to claim 10 is moot as well.

The Rejections Under 35 U.S.C. § 103

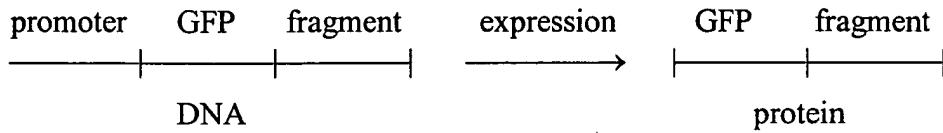
Claims 1-3 and 10 and 13 were rejected as assertedly obvious over Chien, *et al.*, in view of Waldo, *et al.* From the text of the objection, it appears that the inclusion of Waldo is related to claims 4 and 11, which are not included in the original statement of rejection. Waldo is stated to "teach GFP fusion proteins for the formation of folding robustness to improve soluble expression in *E. coli*." Claims 4 and 11 thus appear included therefore in the rejection; claim 7 appears to be included as well (see page 10, end of the bridging paragraph).

Nevertheless, identification of particular claims is perhaps no longer relevant in view of the newly submitted claim set. The basis for rejection can be addressed by contrasting the method of the invention with that of the primary document cited, Chien.

Respectfully, it is difficult for applicants to understand the relevance of Chien to the present invention. Chien describes a method to detect the interaction of two proteins intracellularly by attaching to each a portion of a transcriptional activator protein. Neither portion of the transcriptional activator protein can induce transcription by itself; only when the two proteins containing the two "halves" interact, does the transcriptional activating factor itself come together and become functional. Now that it is functional, it can interact with a reporter system to effect the expression of a reporter gene, such as *lacZ*.

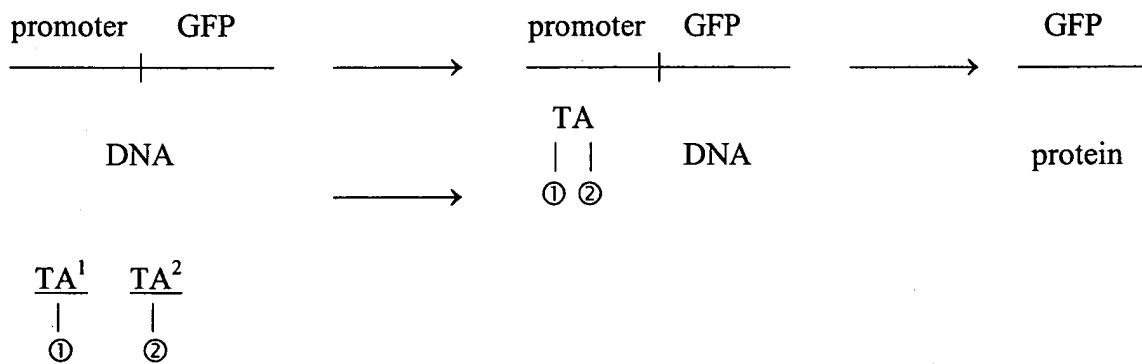
This has nothing to do with the method of the present invention which fuses a functional "reporter" protein to a fragment of a starting protein to be assessed for solubility. Perhaps the following "cartoons" will be helpful, both using, arbitrarily, GFP as the reporter.

Invention Method:



If the protein produced fluoresces, the attached fragment is considered to be a soluble domain; if it does not fluoresce, it is not. The solubility of the domain is roughly proportional to the fluorescence emitted.

Chien:



where

$$\begin{aligned} \text{TA}^1 + \text{TA}^2 &= \text{portions of transcription activator} \\ ① + ② &= \text{interacting proteins} \end{aligned}$$

In a somewhat oversimplified sequence, the two portions of the transcription activator (TA) interact with each other when the proteins to which they are coupled (① and ②) interact, permitting the transcription activator to interact with the promoter resulting in the production of GFP protein.

There appears to be no similarity whatsoever in the two approaches. The first approach is designed to evaluate the solubility of a fragment by evaluating the ability of a fusion of the fragment with GFP to retain its fluorescence; the Chien method is directed to detecting the interaction of two proteins by virtue of their ability to reconstitute a transcription activator thus permitting the production of GFP which, as an isolated protein, will always be fluorescent. Applicants see no connection between these two approaches.

Accordingly, this basis for rejection may properly be withdrawn.

Claims 10-14 were rejected as obvious over Waldo alone.

Waldo is cited in the specification and is at least relevant to the present invention. Waldo prepared fusions of green fluorescent protein with a number of other proteins produced in *E. coli* and observed that the fluorescence emitted by green fluorescent protein is correlated with the appropriate folding of the fused amino acid portion when detached from GFP. Waldo does not suggest applying this approach to identifying soluble domains that are fragments of a starting protein. There is no suggestion in Waldo to obtain fragments of a protein and test them for solubility by fusing the fragments to GFP and detecting fluorescence either *in vivo* or in a cell-free system. Instead, the DNA fused to DNA encoding GFP by Waldo encodes whole proteins or subunits, which are not further treated to form fragments. For example, as shown in figure 1, Waldo expressed 20 different proteins that are fusions with GFP in *E. coli* which include whole enzymes or subunits such as nucleoside diphosphate kinase, tyrosine tRNA kinase, sulfate reductase dissimilatory subunit, and the like. Figures 2 and 3 show only whole or subunit proteins as well. This is unlike the present invention which provides for deletion of a cloned DNA fragment encoding a protein from the 5' or 3' end and expressing the fragment of the

protein. No document has been cited by the Office that makes this suggestion. Accordingly, the rejection over Waldo may also be withdrawn.

CONCLUSION

The rejections under 35 U.S.C. § 112, paragraph 1, appear misplaced as a criticism of claim language rather than rejection for lack of written description or enablement. In any event, the claims have been amended to obviate both this basis for rejection and the rejection made under paragraph 2 of this statutory section. The rejection over Chien appears misplaced, as Chien describes an unrelated method for an unrelated purpose. The rejection over Waldo is in error due to the failure of Waldo to suggest applying fusion techniques to detect soluble domains of, proteins, rather Waldo suggests only evaluation of folding of complete proteins.

Accordingly, it is believed that new claims 15-23 are allowable over the art and passage of these claims to issue is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket No 251002009400.

Respectfully submitted,

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